Schlussbericht zum DZIF-Vorhaben

Vorhabennummer: TI 07.001_008
Vorhabentitel: Clinical leave Programme

Einzelvorhaben

Beteiligte Einrichtung/en sowie Vorhabenlaufzeit/en

Projektkoordination	Laufzeitbeginn		Laufzeitende
Universität Heidelberg	01.03.2023	-	31.03.2024

Verbundpartner	Laufzeitbeginn	Laufzeitende
-		

Kapitel 1: Kurzbericht (wird veröffentlicht)

a) Ursprüngliche Aufgabenstellung und wissenschaftlicher/technischer Stand, an den angeknüpft wurde

The aim of this project was to identify underlying multidimensional shifts in cellular immune composition during halt of the immunosuppressive medication mycophenolic acid (MPA) in a cohort of immunocompromised kidney transplant recipients with a triple immunosuppressive medication who did not seroconvert after ≥3 COVID-19 vaccinations. In addition, we planned to measure the ability of patient immune cells to respond to SARS-CoV-2 infection by co-culturing patient PBMCs with experimentally infected epithelial cells (A549ACE2/TMPRSS2). Unfortunately, the quality of the PBMCs was not sufficient to perform the co-culture experiments as originally planned.
The project was therefore adapted, but the overall goal of studying the host response to SARS-CoV-2 exposure remained. In a large cohort study of 1192 participants aged 4 months to 81 years, we found that the soluble form of the SARS-CoV-2 entry receptor ACE2 was elevated 3 months after SARS-CoV-2 exposure, regardless of confirmed infection. We used a similar experimental design to our original work plan and co-cultured human lung cells with SARS-CoV-2 pseudovirus to determine whether this induced the cells to shed ACE2.

b) Ablauf des Vorhabens

The project was structured into work packages (WPs) and implemented both practically and temporally as follows:

WP1: Immuno-phenotyping (Q2/2023-Q3/2023)
In WP1, the cellular immune composition during discontinuation of MPA and SARS-CoV-2 vaccination was investigated in a cohort of renal transplant recipients who did not seroconvert after ≥3 COVID-19 vaccinations (non-responders).
To assess differences in patient immune function, immune phenotyping using multi-dimensional cytometric profiling was performed on PBMC samples collected at three time points: before discontinuation of MPA (T0), 5 weeks after discontinuation (T1), and 2 months after reintroduction of MPA (T2). All patients were vaccinated with 100 µg mRNA-1273 (Moderna) one week after T0. This analysis was performed in close collaboration with the group of Prof. Stella Autenrieth at the DKFZ Heidelberg using the AURORA device (Cytek) and a comprehensive antibody/fluorophore panel.

WP2: Increased sACE2 activity after SARS-CoV-2 exposure and cocultivation experiments (Q3/23- Q1/24)
Unfortunately, the quality of the PBMCs was not sufficient to perform the cocultivation experiments as originally planned. Instead, we followed up a separate observation made during my clinical leave, finding shedding of the SARS-CoV-2 cell entry receptor ACE2 in SARS-CoV-2 exposed individuals. We replaced WP2's co-culture experiments with an experimental setup to investigate whether shedding of ACE2 and an increase in soluble ACE2 could be reproduced *in vitro* directly by SARS-CoV-2 Spike interaction. Therefore, human lung cells (Calu-3) were incubated with SARS-CoV-2 Spike pseudovirus, cytokines or mock controls. At 16 hours post exposure, supernatants were collected and sACE2 enzyme activity was determined using the commercially available SensoLyte 390 ACE2 activity assay kit.

WP3.1: Data analysis of Immune Cell Phenotyping (Q3/23-Q1/24)
For immune phenotyping, a classical gating strategy was used for quality control of the 36-color panel and unsupervised data analysis using Uniform Manifold Approximation and Projection (UMAP) for dimensionality reduction followed by clustering using self-organized map (FlowSOM). Due to poor quality of PBMCs, technical difficulties and low number of

immune cells in the immunocompromised patients, only 69 samples were included in the final analysis. Therefore, the analysis was kept descriptive.

WP3.2. Data analysis of increase in sACE2 activity after SARS-CoV-2 exposure and cocultivation experiments (Q3/23-Q1/24)
Unpaired and paired t-tests between two groups and ordinary one-way ANOVA with Tukey's correction for multiple comparisons for more than two groups, as well as various multiple linear or logistic regression models, were used to analyze the data from the clinical cohort and the SARS-CoV-2 pseudovirus infection experiments.

c) Wesentliche Ergebnisse und ggf. Zusammenarbeit mit anderen Forschungseinrichtungen

Immune-Phenotyping
Unsupervised data analysis identified 39 distinct immune cell populations and manually assigned specific cell populations.
Differences in the abundance of the 39 cell populations were compared, and differences in cell subpopulations were observed between healthy controls and immunocompromised patients, as well as between immunocompromised patients with MPA and MPA withdrawal, and within the group of patients with MPA withdrawn between different time points.
These results were obtained in close collaboration with the group of Prof. Stella Autenrieth at the DKFZ Heidelberg.

Increased sACE2 activity after SARS-CoV-2 exposure and cocultivation experiments
In a large cohort study of 1192 participants aged 4 months to 81 years, we found that the soluble form of the SARS-CoV-2 entry receptor ACE2 was elevated 3 months after SARS-CoV-2 exposure, regardless of confirmed infection. This finding was confirmed in vitro, and human lung cells exposed to SARS-CoV-2 pseudovirus for 16 hours showed significantly increased sACE2 activity in a dose-dependent manner.

Kapitel 2: Eingehende Darstellung (wird veröffentlicht)

a) Ausführliche Darstellung der erzielten Ergebnisse im Einzelnen

WP1: Immune cell phenotyping

In WP1 we characterized the cellular immune composition during MPA withdrawal and SARS-CoV-2 vaccination in a cohort of kidney transplant recipients with a triple immunosuppressive medication who did not seroconvert after ≥3 COVID-19 vaccinations (nonresponders). For this purpose, we utilized an established and well characterized cohort of 68 longitudinally monitored kidney transplant patients[1]. The transplant cohort was recruited between February 17 and May 18, 2022, at the Department of Nephrology, University of Heidelberg, Germany. Inclusion criteria were: (1) kidney transplant recipients, (2) anti-SARS-CoV-2 specific antibodies of <10 after ≥3 COVID-19 vaccinations (nonresponders) (3) triple immunosuppressive maintenance therapy and (4) stable graft function without graft rejection in the last 12 months. Exclusion criteria were: (1) prior SARS-CoV-2 infection. Mycophenolic acid (MPA) was withdrawn in 43 of 68 patients for 5 weeks. Blood was drawn at three time points: (1) before MPA withdrawal (T0), (2) 5 weeks after MPA withdrawal right before MPA reinduction (T1) and (3) additionally 2 months after MPA reinduction (T2). All patients were vaccinated with 100µg mRNA-1273 by Moderna one week after T0 (4 weeks before T1).

To assess potential differences in patient immune function, we performed immune phenotyping. PBMC samples from patients collected at T0, T1 and T2 and from 6 healthy controls 3-4 weeks after standard SARS-CoV-2 vaccination (T1) were subjected to cytometric profiling. Immune phenotyping was performed through an established close collaboration between the groups of Dr. Binder and Prof. Stella Autenrieth (Research Group "Dendritic Cells in Infection and Cancer", DKFZ, Heidelberg.), who operates an AURORA (Cytek) instrument and has established a comprehensive antibody/fluorophore panel [2]. In detail, PBMCs were thawed rapidly in a 37°C water bath. To remove clumps, 10 ml RPMI medium (Sigma) containing 50 KU DNase I (Merck) was added. The cells were then centrifuged at 400 x g for 5 minutes at room temperature. The supernatant was discarded and the cell pellet was resuspended in 5 mL of fresh RPMI medium containing 200 KU DNase I for 20 minutes at 37°C. Cell viability was then determined by the trypan blue exclusion method using a Neubauer counting chamber. The PBMCs were washed once with PBS and 3 million live cells were isolated for further staining. To exclude dead cells from the analysis, the isolated PBMCs were stained with the LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Thermo Fisher Scientific) according to the manufacturer's instructions (1:1000 dilution). Cell Staining Buffer (BioLegend) was used for all subsequent washing and incubation steps. Non-specific antibody binding to Fc receptors was blocked by incubating cells with Human True Stain FcX (BioLegend) for 10 minutes at room temperature. Extracellular staining was then performed for 1 hour at 4°C. The specific antibodies used for staining are listed in **Table 1**. The staining volume was 100 µl per sample and contained 5 µl True Stain Monocyte Blocker (BioLegend) to prevent non-specific binding of certain fluorochromes (PE/Dazzle594, PE/Cy5, PE/Cy7, APC/Cy7, and APC/Fire750) to monocytes. After staining, the cells were washed twice with staining buffer. Finally, at least 1.5 million live cells were acquired using an Aurora flow cytometer (Cytek Bioscience) with SpectroFlow software. Unmixing, a process to differentiate overlapping fluorescence signals, was performed within SpectroFlow using single-stained controls (cell or bead-based) and unstained cells to account for autofluorescence.

We stained and measured all available samples from the whole cohort as initially planned but unfortunately, a subpar quality of PBMCs, technical difficulties and low numbers of immune cells in the immunocompromised patients resulted in only 69 samples with sufficient quality and data included for final analysis (WP3.1).

Table 1. Antibodies used

Antibody	Company	Catalognumber	Clone	Concentration
CD1c AlexaFluor647	BioLegend	331510	L161	1,25 µg/ml
CD3 BV510	BioLegend	317332	OKT3	0,75 µg/ml
CD4 cFluorYG584	Cytek Biosciences	R7-20041	SK3	0,25 µg/ml
CD8 BUV805	BD Bioscience	612889	SK1	0,8 µg/ml
CD11b PerCP-Cy5.5	BioLegend	301338	ICRF44	5 µg/ml
CD11c BUV661	BD Bioscience	612967	B-ly6	1,25 µl in 100 µl
CD14 Spark Blue 550	BioLegend	367148	63D3	1,6 µg/ml
CD16 BUV496	BD Bioscience	612944	3G8	0,6 µg/ml
CD19 Spark NIR 685	BioLegend	302270	HIB19	0,6 µg/ml
CD20 Pacific Orange	Thermo Fisher Scientific	MHCD2030	HI47	1,25 µl in 100 µl
CD24 PE Dazzle594	BioLegend	311134	ML5	2 µg/ml
CD25 PE	BioLegend	302606	BC96	0,75 µg/ml
CD27 APC	BioLegend	356410	M-T271	2,5 µg/ml
CD28 BV650	BioLegend	302946	CD28.2	1,5 µg/ml
CD38 APC Fire810	BioLegend	102746	HIT2	0,75 µg/ml
CD45 PerCP	BioLegend	368506	2D1	2 µg/ml
CD45RA BUV395	BD Bioscience	740315	5H9	2 µg/ml
CD56 BUV737	BD Bioscience	612766	NCAM16.2	2,5 µl in 100 µl
CD57 FITC	BioLegend	359604	HNK-1	0,6 µg/ml
CD95 PE/Cy5	BioLegend	305610	DX2	1,2 µg/ml
CD123 Super Bright436	Thermo Fisher Scientific	62-1239-42	6H6	0,3 µg/ml
CD127 APC-R700	BD Bioscience	565185	HIL-7R-M21	1,25 µl in 100 µl
CD141 BrilliantBlue515	BD Bioscience	566017	1A4	3 µl from a 1:5 pre-dilution in 100 µl
CD161 eFluor450	Thermo Fisher Scientific	48-1619-42	HP-3G10	1,25 µg/ml
CD183 (CXCR3) PE/Cy7	BioLegend	353720	G025H7	2 µg/ml
CD185 (CXCR5) BV750	BD Bioscience	747111	RF8B2	1,2 µg/ml
CD195 (CCR5) BUV563	BD Bioscience	741401	2D7/CCR5	5 µg/ml

WP3.1: Data analysis and Results of Immune Cell Phenotyping

For **Immune phenotyping** (WP1) we performed a classical gating strategy for quality control of the 36-color panel (**Figure 1**) and unsupervised data analysis (**Figure 2**) using Uniform Manifold Approximation and Projection (UMAP)[3] for dimensionality reduction followed by clustering using self-organized map (FlowSOM)[4] using OMIQ software (Dotmatics, Boston, MA, USA).

Unsupervised data analysis identified 39 distinct immune cell populations and manually assigned specific cell populations (**Figure 2**). These cell populations include a variety of different immune cell types. Already in the UMAP visualization, differences were seen between healthy controls and patients with MPA and MPA withdrawal (**Figure 3**).

Approx. Emission Wavelength (nm)	UV	Violet	Blue	Yellow Green	Red
395	CD45RA BUV395				
420	Viability UV	CCR7 BV421			
440		CD123 SB436			
450		CD161 eFluor450			
480		IgD BV480			
500	CD16 BUV496	CD3 BV510	CD141 BB515		
520			CD57 FITC		
550		CD20 Pacific Orange	CD14 Spark Blue 550		
570	CCR5 BUV563	IgM BV570		CD25 PE	
580				CD4 CF568	
600		IgG BV605		CD24 PE Dazzle594	
660	CD11c BUV661	CD28 BV650			CD27 APC
680			CD45 PerCP	CD95 PE Cy5	CD1c AlexaFluor 647
690			CD11b PerCP Cy5.5		CD19 Spark NIR 685
700		CCR6 BV711	TCR γδ PerCP eFluor710		CD127 APC-R700
730	CD56 BUV737				
750		CXCR5 BV750			
780		PD-1 BV785		CXCR3 PE Cy7	HLA DR APC Fire750
800	CD8 BUV805				CD38 APC Fire 810

T cells
NK cells
B cells
Monocytes
DCs

Figure 1. 36-color antibody panel was used for immune cell phenotyping on a five laser Aurora spectral flow cytometer. Colors depict the marker expression on the indicated cell populations. Panel used for the analysis of immune cell populations

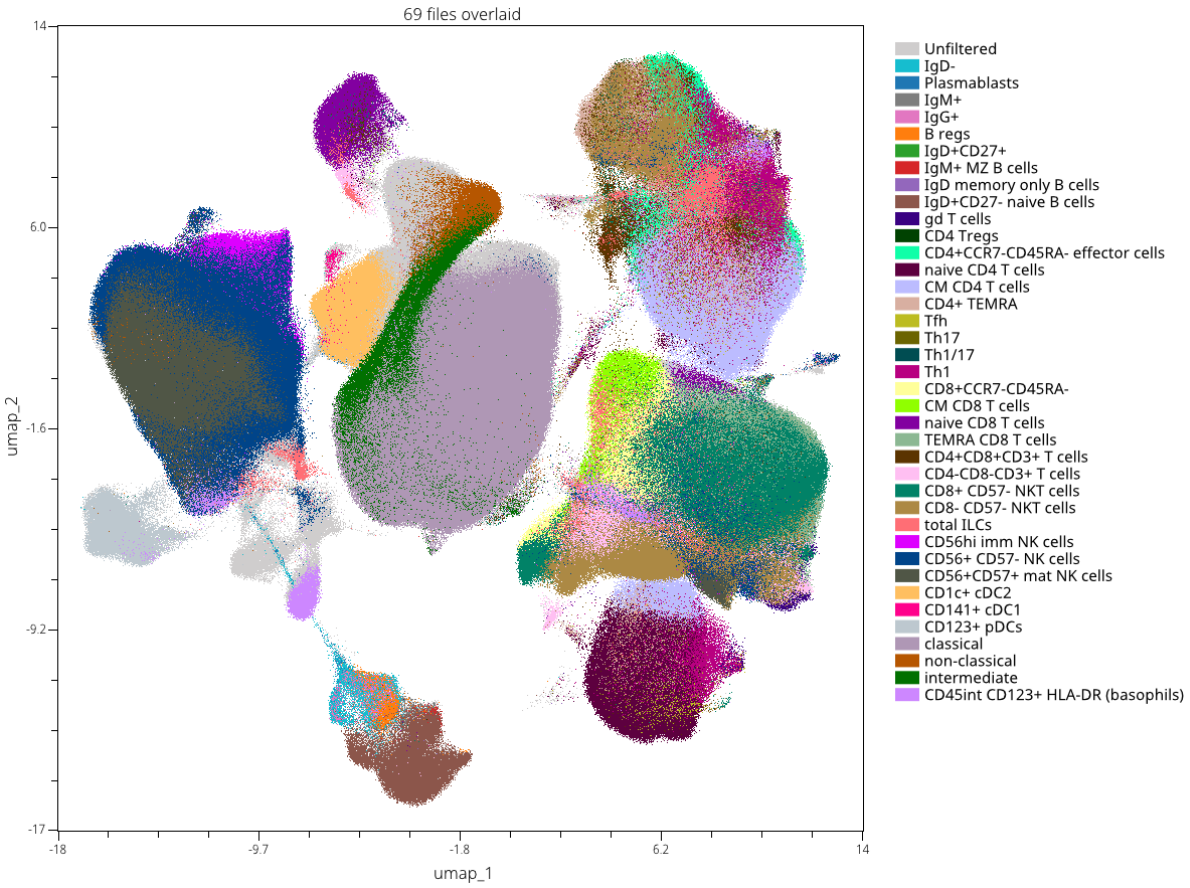

Figure 2. 39 FlowSOM clusters projected onto two UMAP dimensions. The overlay plot shows concatenated events from all samples. FlowSOM clusters were assigned to depicted cell populations based on their marker expression.

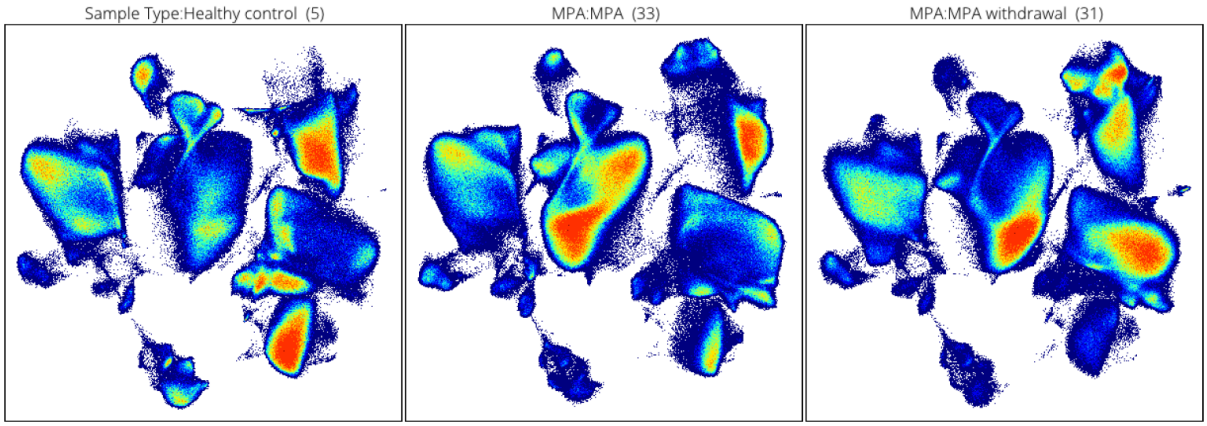

Figure 3. High-dimensional data analysis of PBMCs from healthy controls (n=5 samples), patients with MPA (n=33), and patients with MPA withdrawn (n=31) displayed in two UMAP dimensions. The density plots show concatenated events from all of the indicated samples.

Next, we compared the differences in abundance of the 39 cell populations between

a) healthy controls (blue, good responders) and immunocompromised patients with MPA (orange, MPA) and with MPA withdrawn (green, MPA withdrawal) across all timepoints.
b) healthy controls (blue, good responders) and immunocompromised patients with MPA (orange, MPA) and with MPA withdrawn (green, MPA withdrawal) at T1.
c) before MPA withdrawal (blue, T0), 5 weeks after MPA withdrawal right before MPA reinduction (orange, T1) and additionally 2 months after MPA reinduction (green, T2) in patients with MPA withdrawn.

Due to the low number of samples included and the high variety across samples, we kept the analysis purely descriptive, as the statistical power was not sufficient to answer our original research question. The alternative analysis revealed differences in a variety of immune cell populations between healthy controls and immunocompromised patients with MPA and with MPA withdrawn as well as between the different time points in patients with MPA withdrawn. We aim to publish those results together with colleagues of the Dept. of Nephrology and Immunology.

WP2: Cocultivation experiments

Particularly in case of novel pathogens (such as a pandemic virus), to which no pre-existing adaptive immunity can be expected, swift and strong innate immune responses upon virus encounter are crucial for successful control of the infection [5,6]. To measure the capacity of patients' immune cells to activate and respond to SARS-CoV-2 infection, we planned to co-culture patient PBMCs with experimentally infected epithelial cells (A549ACE2/TMPRSS2). Unfortunately, the quality of the PBMCs was not sufficient to conduct the cocultivation experiments as initially planned.

Therefore, we shifted the focus of the research focus to the role of sACE on the host immune response. One of the key factors contributing to the ability of SARS-CoV-2 to infect and replicate in host cells is its ability and dependence on the host cell receptor angiotensin converting enzyme 2 (ACE2)[7-9]. When ACE2 is shed from cell surface into plasma as soluble ACE2 (sACE2), it can effectively neutralise SARS-CoV-2 (reviewed in[10]). This provides an immune-cell-independent and mutational-resistant broad-spectrum defense mechanism that inhibits SARS-CoV-2 infection locally in the respiratory tract, but also systemically in blood vessels and secondary organs.

SARS-CoV-2-specific B- and T-cell responses may be absent, especially in asymptomatic or mildly infected individuals[11,12]. Children, in particular, more often lack B- and T-cell responses[11,12] but clear SARS-CoV-2 rapidly[13], making it more difficult to detect infections with currently established detection methods. Increased sACE2 concentration and activity have been reported during[14-18] and up to 4 months after [14,19,20] SARS-CoV-2 infection in adults, but no studies have determined sACE2 after asymptomatic SARS-CoV-2 infection,

in children, and especially in participants with proven SARS-CoV-2 household exposure but no evidence of a SARS-CoV-2 infection by current methods of detection.
Following the above described adaptation of the research focus we investigated sACE2 activity longitudinally in a cohort of 1192 participants, aged 4 months to 81 years, 3 and 12 months after SARS-CoV-2 household exposure during the first wave of the pandemic. Following SARS-CoV-2 exposure, participants exhibited significantly elevated sACE2 activity (**Figure 4a**), irrespective of confirmed infection (by PCR, humoral or T-cell immunity) (**Figure 4b**), with the highest levels observed in exposed children (**Figure 5**). Longitudinal analysis revealed a decline in sACE2 levels over time (**Figure 4c)** reaching levels comparable to age- and sex-matched pre-pandemic controls (**Figure 4a**).

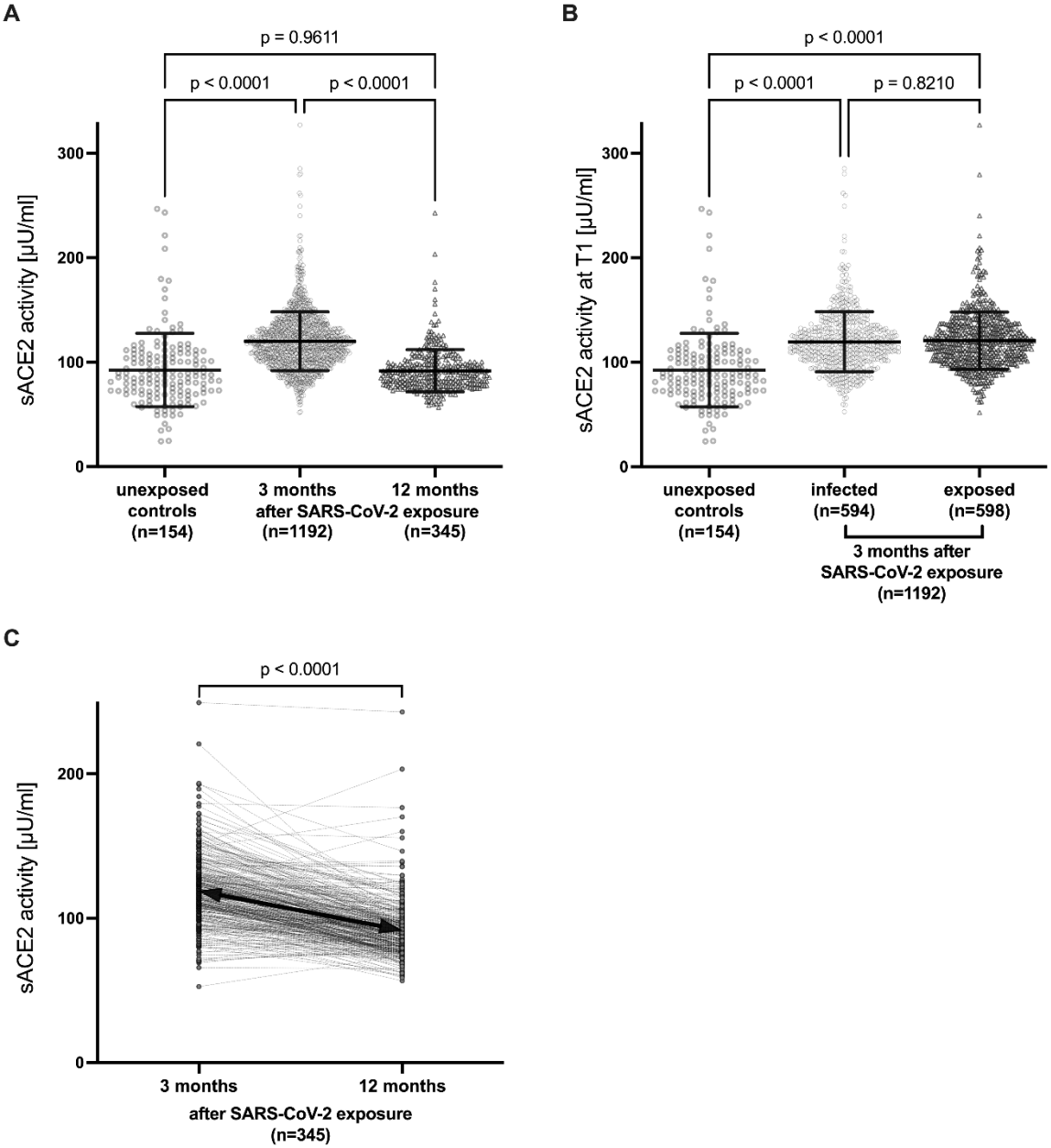

Figure 4. Soluble ACE2 (sACE2) activity in sera from unexposed pre-pandemic controls (n = 154) and **(a)** participants 3 (n=1192) and 12 months (n=345) after SARS-CoV-2 exposure and **(b)** categorized by participants with (n=594) and without (n=598) evidence of infection 3 months after exposure. **(c)** Longitudinal sACE2 activity from 345 participants 3 to 12 months after SARS-CoV-2 exposure. Individual data for each participant are depicted in gray. (a, b) Mean and 1 ± times standard deviation and (c) longitudinal mean are shown in black. p values were calculated using (a, b) one-way ANOVA with Tukey correction for multiple comparisons and (c) paired t-test.

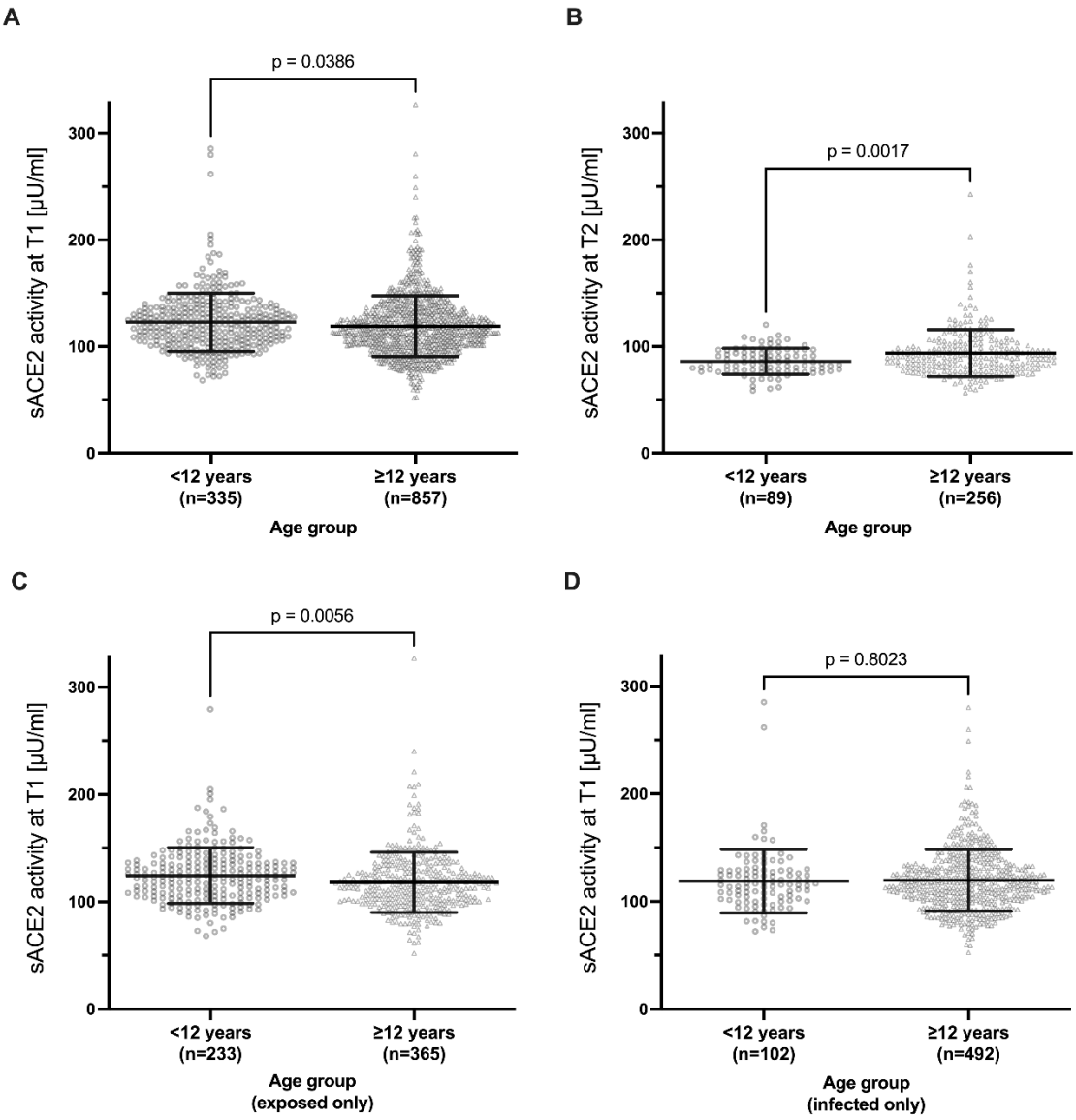

Figure 4. Soluble ACE2 (sACE2) activity stratified by age group in sera of **(a)** 1192 participants 3 months and **(b)** 345 participants 1 year after SARS-CoV-2 exposure and participants **(c)** without and **(d)** with evidence of infection 3 months after SARS-CoV-2 exposure. Mean and 1 ± times standard deviation are shown in black and individual data for each participant are depicted in gray. *p* values were calculated using unpaired t-tests.

To investigate whether ACE2 shedding may be a direct effect of cells being exposed to SARS-CoV-2 Spike, as demonstrated previously for SARS-CoV and HCoV-NL63 [21,22], we incubated human cells with SARS-CoV-2 Spike pseudovirus. In detail, human lung cells (Calu-3) were seeded into a 96-well plate at a density of 30,000 cells per well in 100 µl DMEM supplemented with 20% fetal calf serum (FCS) and 2 mM sodium pyruvate. After 24 hours of incubation, cells were washed with PBS and subsequently exposed to SARS-CoV-2 Spike (S)-pseudotyped lentiviral particles (SCoV-2-Spike), generated using a standard second-generation lentiviral vector protocol with a synthetic codon-optimized SARS-CoV-2 S gene (Wuhan-Hu-1 strain). SCoV-2-Spike pseudovirus were produced in DMEM but then diluted in Opti-MEM to facilitate viral entry. Mock-treated cells serving as a control for non-specific effects, were maintained under identical conditions with corresponding Opti-MEM to DMEM ratios. Additionally, cells were treated with TNF, INF-β or INF-γ (and corresponding mock) to exclude indirect triggering of ACE2 shedding by cytokines. At 16 hours post-exposure, supernatants were collected, and sACE2 enzyme activity was determined using the commercially available SensoLyte 390 ACE2 activity assay kit (72086; AnaSpec, San Jose, CA) according to the manufacturer's protocol. The fluorescence of Mc-Ala was monitored at excitation/emission 330/390 nm.

WP3.2: Data analysis and results of SCoV-2-Spike pseudovirus experiments

ACE2-expressing Calu-3 (human lung) cells exposed to SCoV-2-Spike pseudovirus for 16 hours showed significantly increased sACE2 activity in the supernatant as compared to pseudovirus without SARS-CoV-2 Spike treated cells (Figure 6).

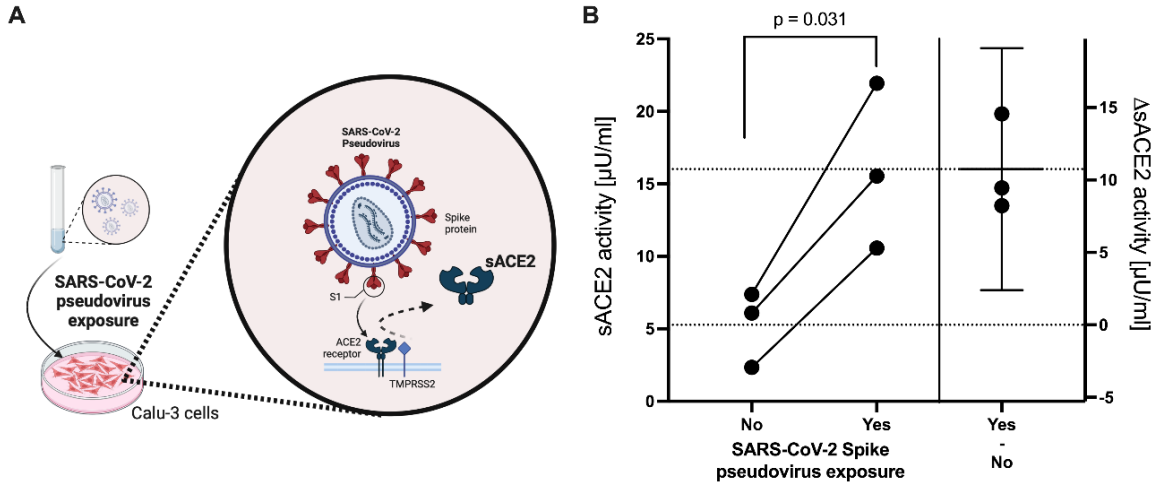

Figure 6. (a) Schematic illustration of *in vitro* experiments to determine soluble ACE2 (sACE2) shedding upon exposure to pseudovirus particles bearing a SARS-CoV-2 Spike protein (SCoV-2 Spike) (created with BioRender.com). **(b)** Absolute sACE2 activity and the change in sACE2 (ΔsACE2) activity in the supernatant of Calu-3 (human lung) cells after 16 hours of exposure to pseudovirus with (yes) vs. without (no) SARS-CoV-2 Spike. Individual data points for each biologically independent experiment are depicted with black dots connected by lines. For ΔsACE2 activity, the mean and confidence intervals among the experiments are shown. *p*-value was calculated using a paired t-test.

In conclusion, this study provides new insights into host responses after SARS-CoV-2 exposure. We observed that sACE2 enzyme activity was elevated after SARS-CoV-2 exposure irrespective of confirmed infection by currently established assays, and we hypothesise that this mechanism may contribute to SARS-CoV-2 clearance. This study therefore challenges the dichotomy of categorising SARS-CoV-2 exposed participants as infected or not infected solely on currently established assays and demonstrates lasting host responses independent of B- and T-cell memory.

b) Meilensteine und Liefergegenstände

Meilensteine

Nr.	Titel	Arbeits-paket	Einrichtung	Datum lt. Vorhaben beschreibung	Korrigiertes Datum	Status	Kommentar/Begründung
1	Immune phenotyping	WP1	Uni-HD	30.07.2023	30.07.2023	Erfüllt	
2	Innate immune function (co-culture)	WP2	Uni-HD	31.09.2023	31.01.2024	Erfüllt	In angepasster Form (siehe WP2)
3	Statistical analysis	WP3	Uni-HD	15.12.2023	31.03.2024	Erfüllt	In angepasster Form
4	Writing of manuscript		Uni-HD	28.02.2023	15.05.2024	Nicht erreichbar	Manuskript geschrieben und aktuell zum final proof bei allen Koautoren

Liefergegenstände

Nr.	Titel	Arbeits-paket	Einrichtung	Datum lt. Vorhaben beschreibung	Korrigiertes Datum	Status	Kommentar/Begründung
1	Raw data after laboratory analysis	WP1-2	Uni-HD	15.11.2023	31.01.2023	Erfüllt	In angepasster Form
2	Comprehensive analysis of data	WP3	Uni-HD	15.12.2023	31.03.2024	Erfüllt	In angepasster Form
3	Submission of manuscript		Uni-HD	28.02.2024		Nicht erreichbar	Manuskript geschrieben und aktuell zum final proof bei allen Koautoren

c) Wichtigste Positionen des zahlenmäßigen Nachweises

Personalmittel

Einsatz: Physician scientist (TV-Ä2, 100%);04/2023-03/2024

Sachmittel (auch Aufträge)

The consumables were used as planned for the successful execution of the project.

d) Notwendigkeit und Angemessenheit der geleisteten Arbeiten

All costs mentioned above were within the financial frame of the DZIF clinical leave grant Basal funding (including lab space and equipment) were provided by the institution. However, the project required funding for project specific consumables and the salary of the physician scientist. The incurring expenses could not be covered through existing funding of the group, and currently, no alternative funding sources exist. Without the DZIF funding we would not have been able to carry out the abovementioned experiments.

e) Voraussichtlicher Nutzen des Vorhabens, insb. die Verwertbarkeit des Ergebnisses im Sinne des fortgeschriebenen Verwertungsplans

Collaboration and Knowledge Sharing:

- **Open Access Publications:** We will publish our findings in open-access journals to ensure broad dissemination and accessibility to the global scientific community.
- **Research Consortia:** We will actively seek out and present the research in workshops or consortia focused on SARS-CoV-2 and other infectious diseases. This will facilitate the exchange of expertise, data, and resources to accelerate progress.
- **Public Health Engagement:** Relying solely on antibodies provides an incomplete picture of immune response. Our research demonstrates the existence of lasting host responses independent of B- and T-cell memory, which might contribute to virus clearance. Excluding individuals based solely on antibody status could overlook this vital aspect of immunity and might disadvantage certain populations, such as children who more often lack an antibody response despite mounting an effective immune defense.

Target Audience for Findings:

Our findings are relevant to a wide range of stakeholders:

- **Scientific Community:** Researchers studying SARS-CoV-2, immunology, and infectious diseases can utilize our findings to advance their understanding of the virus and develop new therapeutic strategies.
- **Public Health Agencies:** Public health officials can use our findings to improve surveillance, risk assessment, and public health policies related to SARS-CoV-2 and other infectious diseases in the future.

Concrete Plans for Near-Future Utilization and Collaboration

- **Unveiling the Role of Virus entry receptor shedding as an early defense mechanism:** Our findings might suggest a link between elevated sACE2 as a rapid host mechanism. Further exploration in this area could lead to a better understanding of the body's early defense mechanisms against SARS-CoV-2 and potentially other viruses. This knowledge could be valuable for developing novel therapeutic strategies.

- **Generalizability of the Entry Receptor Shedding Concept:** Our initial observations suggest that the shedding of the virus entry receptor might be a broader host mechanism. Investigating this concept across different viruses could be an interesting area of research.

By implementing these plans and actively engaging with stakeholders, we can ensure that our research findings have a lasting impact on scientific understanding and public health.

f) Während der Durchführung des Vorhabens dem Zuwendungsempfänger bekannt gewordener Fortschritt auf dem Gebiet des Vorhabens bei anderen Stellen

NA

g) Erfolgte oder geplante Veröffentlichung der Ergebnisse nach Nr. 5 der Nebenbestimmungen für Zuwendungen (NABF/NKBF 2017)

- Maximilian Stich, Vladimir Gonçalves Magalhães, Friederike Bürger, Sven F. Garbade, Kathrin Jeltsch, Kerstin Mohr, Anneke Haddad, Roland Elling, Peter Lang, Armin Rabsteyn, Eva-Maria Jacobsen, Sebastian Bode, Barbara Müller, Hans-Georg Kräusslich, Georg Friedrich Hoffmann, Jürgen Okun, Ralf Bartenschlager, Marco Binder, Aleš Janda, Hanna Renk, Burkhard Tönshoff. Elevated Soluble ACE2 Activity in Children and Adults After SARS-CoV-2 Exposure Irrespective of Laboratory-Confirmed Infection. J Med Virol. 2024 Dec;96(12):e70098. doi: 10.1002/jmv.70098. PMID: 39624009; PMCID: PMC11612704.

References

1. Kühn T, Speer C, Morath C, et al. Immune Response to COVID-19 mRNA Vaccination in Previous Nonresponder Kidney Transplant Recipients After Short-term Withdrawal of Mycophenolic Acid 1 and 3 Months After an Additional Vaccine Dose. Transplantation 2023;107(5) (https://journals.lww.com/transplantjournal/Fulltext/2023/05000/Immune_Response_to_COVID_19_mRNA_Vaccination_in.23.aspx).
2. Friedrich M, Hahn M, Michel J, et al. Dysfunctional dendritic cells limit antigen-specific T cell response in glioma. Neuro Oncol 2022 (In eng). DOI: 10.1093/neuonc/noac138.
3. McInnes L, Healy J. UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction. ArXiv 2018;abs/1802.03426.
4. Van Gassen S, Callebaut B, Van Helden MJ, et al. FlowSOM: Using self-organizing maps for visualization and interpretation of cytometry data. Cytometry Part A 2015;87(7):636-645. DOI: https://doi.org/10.1002/cyto.a.22625.
5. Loske J, Röhmel J, Lukassen S, et al. Pre-activated antiviral innate immunity in the upper airways controls early SARS-CoV-2 infection in children. Nature Biotechnology 2022;40(3):319-324. DOI: 10.1038/s41587-021-01037-9.
6. Ahmed R, Oldstone MB, Palese P. Protective immunity and susceptibility to infectious diseases: lessons from the 1918 influenza pandemic. Nat Immunol 2007;8(11):1188-93. (In eng). DOI: 10.1038/ni1530.
7. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579(7798):270-273. (In eng). DOI: 10.1038/s41586-020-2012-7.
8. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 2020;181(2):271-280.e8. (In eng). DOI: 10.1016/j.cell.2020.02.052.
9. Tai W, He L, Zhang X, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a

viral attachment inhibitor and vaccine. Cell Mol Immunol 2020;17(6):613-620. (In eng). DOI: 10.1038/s41423-020-0400-4.
10. Zhang H, Lv P, Jiang J, et al. Advances in developing ACE2 derivatives against SARS-CoV-2. Lancet Microbe 2023;4(5):e369-e378. (In eng). DOI: 10.1016/s2666-5247(23)00011-3.
11. Dulovic A, Rabsteyn A, Remppis J, et al. Longitudinal Analysis of Humoral and Cellular Immunity in SARS-CoV-2 Exposed Families. SSRN; 2023.
12. Khoo WH, Jackson K, Phetsouphanh C, et al. Tracking the clonal dynamics of SARS-CoV-2-specific T cells in children and adults with mild/asymptomatic COVID-19. Clin Immunol 2023;246:109209. (In eng). DOI: 10.1016/j.clim.2022.109209.
13. Costa R, Bueno F, Albert E, et al. Upper respiratory tract SARS-CoV-2 RNA loads in symptomatic and asymptomatic children and adults. Clin Microbiol Infect 2021;27(12):1858.e1-1858.e7. (In eng). DOI: 10.1016/j.cmi.2021.08.001.
14. Lundström A, Ziegler L, Havervall S, et al. Soluble angiotensin-converting enzyme 2 is transiently elevated in COVID-19 and correlates with specific inflammatory and endothelial markers. Journal of Medical Virology 2021;93(10):5908-5916. DOI: https://doi.org/10.1002/jmv.27144.
15. Kragstrup TW, Singh HS, Grundberg I, et al. Plasma ACE2 predicts outcome of COVID-19 in hospitalized patients. PloS one 2021;16(6):e0252799-e0252799. (In eng). DOI: 10.1371/journal.pone.0252799.
16. Vassiliou AG, Zacharis A, Keskinidou C, et al. Soluble Angiotensin Converting Enzyme 2 (ACE2) Is Upregulated and Soluble Endothelial Nitric Oxide Synthase (eNOS) Is Downregulated in COVID-19-induced Acute Respiratory Distress Syndrome (ARDS). Pharmaceuticals (Basel) 2021;14(7) (In eng). DOI: 10.3390/ph14070695.
17. Nagy B, Jr., Fejes Z, Szentkereszty Z, et al. A dramatic rise in serum ACE2 activity in a critically ill COVID-19 patient. Int J Infect Dis 2021;103:412-414. (In eng). DOI: 10.1016/j.ijid.2020.11.184.
18. Fagyas M, Fejes Z, Sütő R, et al. Circulating ACE2 activity predicts mortality and disease severity in hospitalized COVID-19 patients. Int J Infect Dis 2022;115:8-16. (In eng). DOI: 10.1016/j.ijid.2021.11.028.
19. Phetsouphanh C, Darley DR, Wilson DB, et al. Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection. Nat Immunol 2022;23(2):210-216. (In eng). DOI: 10.1038/s41590-021-01113-x.
20. Patel SK, Juno JA, Lee WS, et al. Plasma ACE2 activity is persistently elevated following SARS-CoV-2 infection: implications for COVID-19 pathogenesis and consequences. Eur Respir J 2021;57(5) (In eng). DOI: 10.1183/13993003.03730-2020.
21. Haga S, Yamamoto N, Nakai-Murakami C, et al. Modulation of TNF-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-alpha production and facilitates viral entry. Proc Natl Acad Sci U S A 2008;105(22):7809-14. (In eng). DOI: 10.1073/pnas.0711241105.
22. Glowacka I, Bertram S, Herzog P, et al. Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63. J Virol 2010;84(2):1198-205. (In eng). DOI: 10.1128/jvi.01248-09.